Lysostaphin

Week 4

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Digestion of Lysostaphin from plasmid Biobrick

Responsible

Bethel Tesfai Embaie

Protocols used

3A Assembly
Gel electrophoresis

Experimental Set Up

Lysostaphin PCR product 101.3ng/μl

Table 1: Samples and digestions. sd is single digestion and dd is double digestion.

Label	Digestion
L1	sd XbaI
L2	sd PstI
L3	dd
L4	dd
L5	dd
L6	undigested negative control

Table 2: Enzyme Master Mixes for Lysostaphin and Nuc digestion

Enzyme MM XbaI	Volume [μl]	**Enzyme MM PstI**	Volume [μl]
Total Volume	16	Total Volume	16
10x cutsmart	1.6	10x cutsmart	1.6
ddH_20	13.6	ddH_20	13.6
XbaI enzyme	0.8	PstI enzyme	0.8

Table 3: Materials needed for the single digestion

Single digestion	Volume (μl)
DNA sample	0.99
Enzyme MasterMix	2
10x cutsmart	0.6
ddH_20	4.41

Results and Conclusions

Discussion and Troubleshooting

As can be seen in Figure 1. the single digestions is hard to tell if they were positive. The double digestion in well 10-12 differed and all three samples was gel extracted. The result can be seen

Table 4: Material needed for the double digestions

Double digestion	Volume [μl]
DNA sample	0.99
Enzyme MasterMix 1	2
Enzyme MasterMix 2	2
10x cutsmart	0.4
ddH_20	2.61

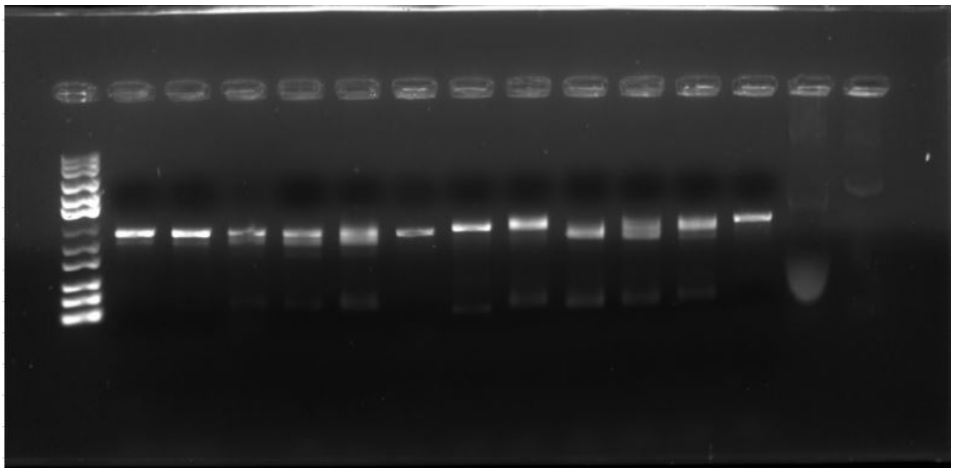

Figure 1: Well 1 is the DNA ladder, 2-7 is not of interest, 8-13 follows the same pattern as Table 1.

below in section 2.

2 Gel extraction of double digested product

Responsible

Bethel Tesfai Embaie

Protocols used

Gel extraction

Modifications to protocols

Used prepared Isopropanol from fume-hood, Performed step 6, Let QG buffer stand for a minute, 25μl EB elution buffer and left to stand for 4 minutes.

Results and Conclusions

Table 5: Volumes used for gel extraction

Gel sample	**Ly 3**	**Ly 4**	**Ly 5**
Weighed each gel:	307.0 mg	315.6 mg	291.2 mg
Added QG buffer 3x vol:	921 μl	946.8 μl	873.6 μl
Isopropanol 1 volume:	307.0 μl	315.6 μl	291.2 μl

The final concentration after the gel extraction was measured with nanodrop to 5.1 ng/μl.

Discussion and Troubleshooting

The sample was saved and used for further research.

3 3A Assembly of Lys

Responsible

Bethel Tesfai Embaie

Protocols used

3A assembly

Experimental Set Up

Table 6: Set up for ligation

General Parameters		
Concentration DNA sample 1 (T7)	6.52	ng/μl
Ratio of vector:insert 1 (molar!)	3	x more insert
Concentration DNA sample 2 (Lys)	5.1	ng/μl
Ratio of vector:insert 2 (molar!)	3	x more insert
Concentration DNA vector	2.7	ng/μl
Size of vector (bp)	2200	bp
Amount of vector per reaction	50	ng
Amount of vector per reaction (mol)	0.000000000000036777	moles
Amount of vector per reaction	18.52	μl
Amount of T4 DNA Ligase	1	μl
Total reaction volume	40	μl

Table 7: Amount of DNA used for the ligation

Sample	**T7**	**Lys**
Size of insert (bp)	55	741
Amont of DNA	0.5757479929 μl	9.91 μl

Table 8: Amount of each material for the ligation

Reaction 1	**Amount (μl)**
DNA sample 1 (T7)	0.58
DNA sample 2 (Lys)	9.91
DNA vector	18.52
T4 Ligase	1
10x T4 DNA Ligase Buffer	4
ddH_20	6.00

Ligated at RT for 20 min, heat kill 80 °C/20 min.

Results and Conclusions

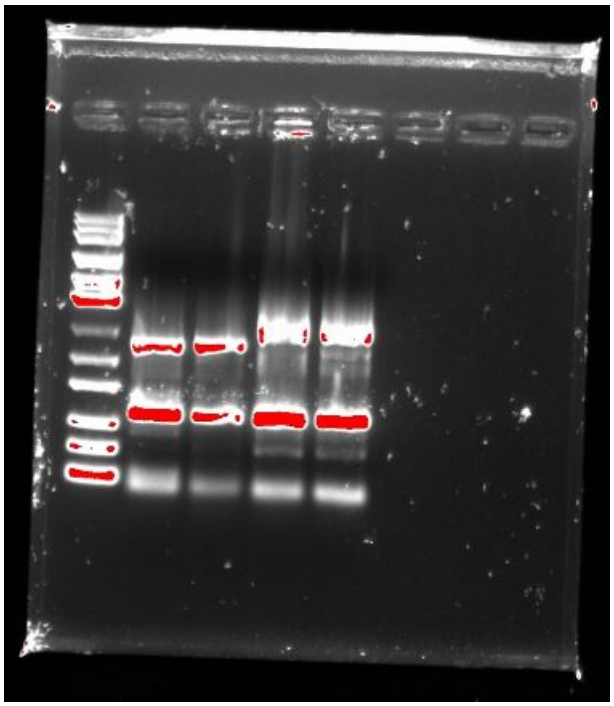

Figure 2: Well 1 is the DNA ladder, 2-3 is not of interest, well 4 is Lys ligation product and well 5 is negative control.

Discussion and Troubleshooting

The size of the ligation product was higher than 765 bp which it should have been. This was misread and the samples was used for further research even though it was not a succeeded 3A Assembly product.

4 Transformation into TOP10

Responsible

Aman Mebrahtu

Protocols used

Transformation
Colony picking

Experimental Set Up

50ul TOP10 KCM-competent cells were transformed with 5μl of ligation product from EC4 and EB5 as well as 3μl RFP control plasmid (50ng/μl) and 100 μl SOC was added to each sample. Because of technical issues, samples were first incubated for 20 min at approximately 25C and 150 rpm before being moved to 37C, 150 rpm where they were left to recover for a full hour. Of the 150 μl cell suspension, 135ul were plated onto a kanamycin plate (35μg/ml Kanamycin) or chloramphenicol plate (35μg/ml Chloramphenicol, only for cells transformed with RFP). The remaining 15μl were plated onto an agar plate without antibiotics as negative control

Results and Conclusions

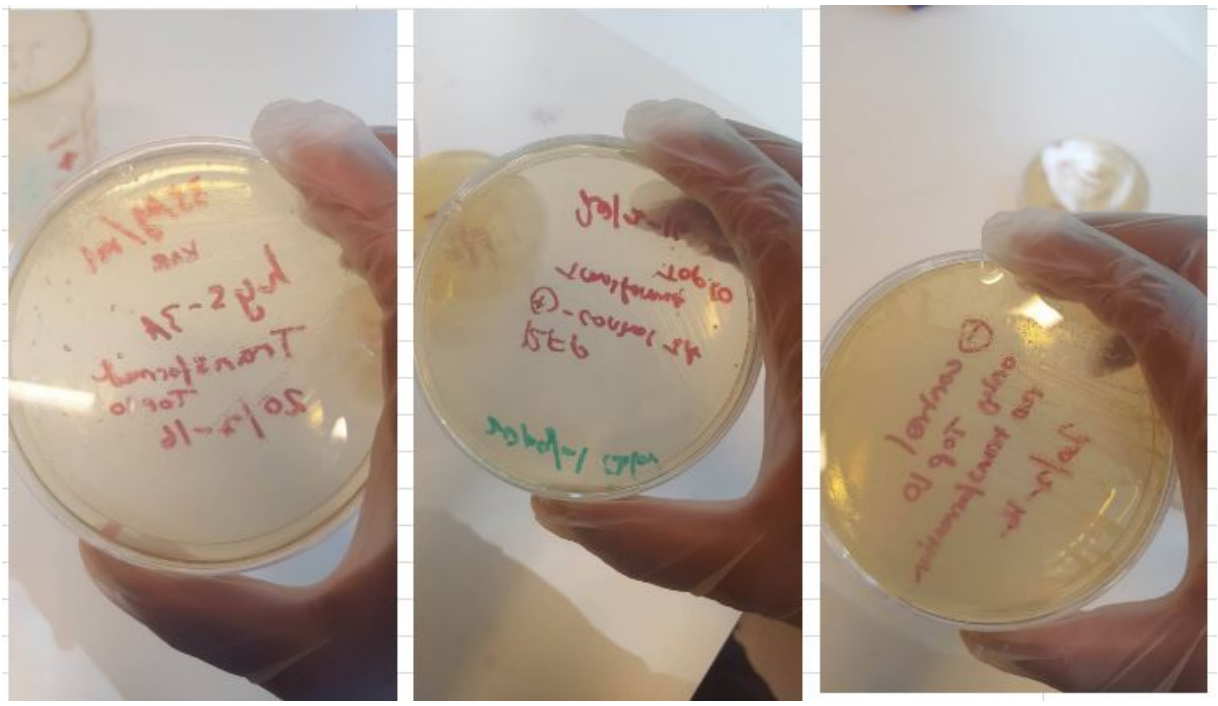

Figure 3: To the left is Lys 1 (many colonies), middle one is positive control and third one is negative control.

Discussion and Troubleshooting

Three colonies were picked from the Lys 1 plate in Figure 3. These were taken during colony picking and colony PCR in section 5.

5 Colony picking & PCR

Responsible

Bethel Tesfai Embaie

Protocols used

PCR Amplification (Q5)

Experimental set up

PCR was performed in a total volume of 25 ul. Liquid cultures were growing in E-flasks and volume was adjusted to correspond to a third of the flasks' volumes: 3 ml LB medium and 3 ul Kanamycin 35 μg/ml 3 colonies of Nuc2 and 3 colonies of Lys1 were grown incubated tubes overnight on the shaker (37 °C, 180 rpm)

Table 9: Amount used for PCR

Total Volume:	25 [μl]
2X PCR Master Mix	12.5
Forward primer	3
Reverse primer	3
ddH_20	6.5
Sample DNA	inoculated

Result and conclusions

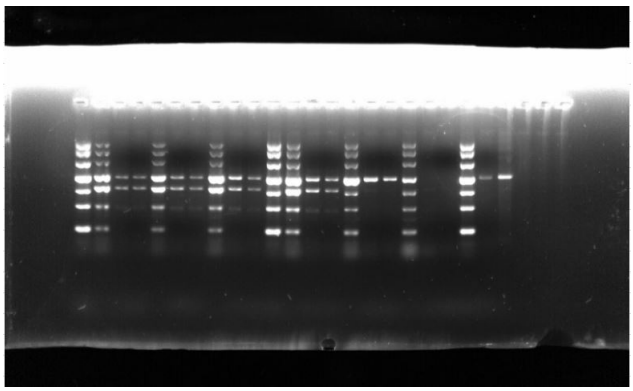

Figure 4: Result from gel electrophoresis. Multiple wells have ladder contamination. The formation can be seen in Table 10

Table 10: Formation of the wells in Figure 4

Well	Sample	Dilution	Information
1	DNA Ladder		
2-10	Nuc		
11	DNA Ladder		
12	Lys 1.1	1:1	Ladder contamination
13	Lys 1.1	1:5	
14	Lys 1.1	1:10	
15	Lys 1.2	1:1	Ladder contamination
16	Lys 1.2	1:5	
17	Lys 1.2	1:10	
18	Lys 1.3	1:1	Ladder contamination
19	Lys 1.3	1:5	
20	Lys 1.3	1:10	
21	DNA Ladder		
22-23	EB		

Discussion and Troubleshooting

The result from the gel electrophoresis shows multiple contaminations and the wrong sizes. The Lys samples were probably contaminated with Eb. These samples was discarded.